

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for selective culturing of primary cell cultures comprising culturing tissue biopsies in the presence of at least 25% serum relative to the amount of culture medium.
2. A method according to claim 1, wherein the serum is between about 25% to about 70%.
3. A method according to claim 1, wherein the serum is between about 30% to about 50%.
4. A method according to claim 1, wherein the serum is between about 30%.
5. A tissue-culture media composition for the selective culturing of primary cell cultures comprising about 30% serum and about 70% culture medium.
6. A tissue-culture media composition according to claim 5, wherein the culture medium is selected from the group consisting of Synthetic Oviductal Fluid (SOF), Modified Eagle's Medium (MEM), Dulbecco's Modified Eagle's Medium (DMEM), RPMI 1640, F-12, IMDM, Alpha Medium and McCoy's Medium.
7. A tissue-culture media composition according to claim 5 or claim 6, wherein the serum is selected from the group consisting of allogeneic serum, autologous serum AND xenogeneic serum.
8. A tissue-culture media composition according to claim 5 or claim 6, wherein the serum is heat-inactivated autologous serum.

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9. A tissue-culture media composition according to any one of claims 5 to 8, further comprising growth factors, co-factors, salts or antibiotics.

5 10. A method for selective culturing of primary cell cultures comprising:

(i) obtaining a tissue biopsy from an animal;

(ii) culturing said tissue biopsy in tissue culture medium comprising at least 25% serum; and

10 (iii) replacing about 50% of the culture medium including serum about every 48 hours.

11. A method according to claim 10, wherein the tissue biopsies are cultured in the presence of a feeder
15 cell layer.

12. A method according to claim 11, wherein the feeder cell layer comprises cultured autologous cells.

20 13. A method according to any one of claims 10 to 12, wherein the tissue biopsies are obtained from a mammalian animal.

14. A method according to claim 13, wherein the
25 mammalian animal is selected from the group consisting of platypus, echidna, kangaroo, wallaby, shrews, moles, hedgehogs, tree shrews, elephant shrews, bats, primates (including chimpanzees, gorillas, orang-utans, humans), edentates, sloths, armadillos, anteaters, pangolins,
30 rabbits, picas, rodents, whales, dolphins, porpoises, carnivores, aardvark, elephants, hyraxes, dugongs, manatees, horses, rhinos, tapirs, antelope, giraffe, cows or bulls, bison, buffalo, sheep, big-horn sheep, horses, ponies, donkeys, mule, deer, elk, caribou, goat, water
35 buffalo, camels, llama, alpaca, pigs and hippos.

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15. A method according to claim 13, wherein the tissue biopsies are isolated from an ungulate selected from the group consisting of domestic or wild bovid, ovid, cervid, suid, equid and camelid.

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16. A method according to claim 13, wherein the tissue biopsies are isolated from a human subject.

10 17. A method according to any one of claims 13 to 16, wherein the tissue biopsies are obtained from an organ selected from the group consisting of skin, lung, pancreas, liver, stomach, intestine, heart, reproductive organs, bladder, kidney urethra and other urinary organs.

15 18. A method according to claim 17, wherein the tissue biopsies are obtained from fetal tissue.

19. A method according to claim 17, wherein the tissue biopsies are obtained from adult tissue.

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20. An isolated tissue-specific progenitor cell or stem cell-like cell obtained by a method according to any one of claims 1 to 19.

25 21. An isolated tissue-specific progenitor cell according to claim 20, wherein the cell is a mesenchymal connective tissue-derived stem cell.

30 22. An isolated mesenchymal connective tissue-derived stem cell.

23. An isolated mesenchymal connective tissue-derived stem cell according to claim 22, wherein the cell has the capacity to be induced to differentiate to form at least one differentiated cell type of mesodermal, ectodermal and endodermal origin.

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24. A cell according to claim 22 or claim 23, wherein said cell is derived from a non-embryonic organ or tissue of a mammal.

5 25. A cell according to any one of claims 22 to 24, wherein the cell has the capacity to be induced to differentiate to form cells selected from the group consisting of osteoblast, chondrocyte, adipocyte, fibroblast, marrow stroma, skeletal muscle, smooth muscle,
10 cardiac muscle, occular, endothelial, epithelial, hepatic, pancreatic, hematopoietic, glial, neuronal and oligodendrocyte cell type.

15 26. A cell according to claim 24, wherein the organ or tissue is selected from the group consisting of bone marrow, muscle, brain, umbilical cord blood and placenta.

20 27. A cell according to any one of claims 24 to 27, wherein the mammal is a human.

28. A cell according to any one of claims 23 to 27, wherein differentiation is induced *in vivo* or *ex vivo*.

25 29. A cell according to any one of claims 22 to 28, wherein the cell constitutively expresses oct4 and high levels of telomerase.

30 30. An isolated mesenchymal connective tissue-derived stem cell as deposited under the Budapest Treaty at the Deutsche Sammlung Von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany on September 2004, under accession number #12345.

35 31. A method of creating a normal non-human animal comprising the steps of: (a) introducing a mesenchymal connective tissue-derived stem cell into a blastocyst; (b)

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implanting the blastocyst of (a) into a surrogate mother;
and (c) allowing the pups to develop and be born.

32. A method according to claim 31, wherein the
5 normal non-human animal is a chimeric animal.

33. A composition comprising a population of a
mesenchymal connective tissue-derived stem cell and a
culture medium, wherein the culture medium expands the
10 mesenchymal connective tissue-derived stem cells.

34. A composition according to claim 33, wherein the
culture medium comprises epidermal growth factor (EGF) and
platelet derived growth factor (PDGF).
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35. A composition according to claim 34, wherein the
culture medium further comprises leukemia inhibitory
factor (LIF).

20 36. A composition comprising a population of fully
or partially purified a mesenchymal connective tissue-
derived stem cell progeny.

37. A composition according to claim 36, wherein the
25 progeny have the capacity to be further differentiated.

38. A composition according to claim 36, wherein the
progeny have the capacity to terminally differentiate.

30 39. A composition according to claim 36, wherein the
progeny are of the osteoblast, chondrocyte, adipocyte,
fibroblast, marrow stroma, skeletal muscle, smooth muscle,
cardiac muscle, ocular, endothelial, epithelial, hepatic,
pancreatic, hematopoietic, glial, neuronal or
35 oligodendrocyte cell type.

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40. A method for isolating and propagating a mesenchymal connective tissue-derived stem cell comprising the steps of: (a) obtaining tissue from a mammal; (b) establishing a population of adherent cells; (c) recovering said mesenchymal connective tissue-derived stem cells; and (d) culturing mesenchymal connective tissue-derived stem cells under expansion conditions to produce an expanded cell population.

41. An expanded cell population obtained by a method according to claim 40.

42. A method for differentiating mesenchymal connective tissue-derived stem cells *ex vivo* comprising the steps of (a) obtaining tissue from a mammal; (b) establishing a population of adherent cells; (c) recovering said mesenchymal connective tissue-derived stem cells; (d) culturing mesenchymal connective tissue-derived stem cells under expansion conditions to produce an expanded cell population and (e) culturing the propagated cells in the presence of desired differentiation factors.

43. A method according to claim 42, wherein the differentiation factors are selected from the group consisting of basic fibroblast growth factor (bFGF); vascular endothelial growth factor (VEGF); dimethylsulfoxide (DMSO) and isoproterenol; and, fibroblast growth factor4 (FGF4) and hepatocyte growth factor (HGF).

44. A method according to claim 42, wherein the differentiated cell obtained by said method is ectoderm, mesoderm or endoderm.

45. A method according to claim 42, wherein the differentiated cell obtained by said method is of the osteoblast, chondrocyte, adipocyte, fibroblast, marrow

stroma, skeletal muscle, smooth muscle, cardiac muscle, ocular, endothelial, epithelial, hepatic, pancreatic, hematopoietic, glial, neuronal or oligodendrocyte cell type.

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46. A method for differentiating a mesenchymal connective tissue-derived stem cell *in vivo* comprising the steps of (a) obtaining tissue from a mammal; (b) establishing a population of adherent cells; (c) recovering said mesenchymal connective tissue-derived stem cells; (d) culturing mesenchymal connective tissue-derived stem cells under expansion conditions to produce an expanded cell population and (e) administering the expanded cell population to a mammalian host, wherein said cell population is engrafted and differentiated *in vivo* in tissue specific cells, such that the function of a cell or organ, defective due to injury, genetic disease, acquired disease or iatrogenic treatments, is augmented, reconstituted or provided for the first time.

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47. A method according to claim 46, wherein the tissue specific cells are of the osteoblast, chondrocyte, adipocyte, fibroblast, marrow stroma, skeletal muscle, smooth muscle, cardiac muscle, ocular, endothelial, epithelial, hepatic, pancreatic, hematopoietic, glial, neuronal or oligodendrocyte cell type.

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48. A method according to claim 46 or claim 47, wherein the mesenchymal connective tissue-derived stem cell undergoes self-renewal *in vivo*.

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49. A method according to any one of claims 46 to 48, wherein cells are administered in conjunction with a pharmaceutically acceptable matrix.

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50. A method according to claim 49, wherein the matrix is biodegradable.

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51. A method according to any one of claims 46 to 50, wherein administration is via localized injection, systemic injection, parenteral administration, oral
5 administration, or intrauterine injection into an embryo.

52. A method according to claim 51, wherein localized injection comprises catheter administration.

10 53. A method according to any one of claims 46 to 52, wherein the disease is selected from the group consisting of cancer, cardiovascular disease, metabolic disease, liver disease, diabetes, hepatitis, hemophilia, degenerative or traumatic neurological conditions,
15 autoimmune disease, genetic deficiency, connective tissue disorders, anemia, infectious disease and transplant rejection.

54. A differentiated cell obtained by a method
20 according to any one of claims 46 to 53.

55. A method of treatment comprising administering to an animal in need thereof a therapeutically effective amount of a cell according to claim 54.

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56. A method according to claim 55, wherein no teratomas are formed in the animal.

57. A method of treatment comprising administering
30 to an animal in need thereof a therapeutically effective amount of mesenchymal connective tissue-derived stem cells or their progeny.

58. A method according to claim 57, wherein reduced
35 or no pretreatment of the animal is required.

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59. A method according to claim 58, wherein pretreatment comprises myeloablation via irradiation or chemotherapy.

5 60. A method according to claim 57, wherein post immunosuppressive treatment of the patient is reduced compared with traditional pharmacological doses.

10 61. A method according to any one of claims 57 to 60, wherein the progeny have the capacity to be further differentiated.

15 62. A method according to claim 61, wherein the progeny are terminally differentiated.

63. A method according to any one of claims 57 to 62, wherein the mesenchymal connective tissue-derived stem cells or their progeny are administered via localized injection, systemic injection, parenteral administration, 20 oral administration, or intrauterine injection into an embryo.

64. A method according to claim 63, wherein localized injection comprises catheter administration.

25 65. A method according to any one of claims 57 to 64, wherein cells are administered in conjunction with a pharmaceutically acceptable matrix.

30 66. A method according to claim 65, wherein the matrix is biodegradable.

67. A method according to any one of claims 57 to 66, wherein the mesenchymal connective tissue-derived stem 35 cells or their progeny alter the immune system to resist viral, bacterial or fungal infection.

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68. A method according to any one of claims 57 to 66, wherein the mesenchymal connective tissue-derived stem cells or their progeny augment, reconstitute or provide for the first time the function of a cell or organ defective due to injury, genetic disease, acquired disease or iatrogenic treatments.

69. A method according to claim 68, wherein the organ is selected from the group consisting of bone marrow, blood, spleen, liver, lung, intestinal tract, eye, brain, immune system, circulatory system, bone, connective tissue, muscle, heart, blood vessels, pancreas, central nervous system, peripheral nervous system, kidney, bladder, skin, epithelial appendages, breast-mammary glands, fat tissue, and mucosal surfaces including oral esophageal, vaginal and anal.

70. A method according to any one of claims 57 to 69, wherein the mesenchymal connective tissue-derived stem cells or their progeny undergo self-renewal *in vivo*.

71. A method according to claim 68, wherein the disease is selected from the group consisting of cancer, cardiovascular disease, metabolic disease, liver disease, diabetes, hepatitis, hemophilia, degenerative or traumatic neurological conditions, autoimmune disease, genetic deficiency, connective tissue disorders, anemia, infectious disease and transplant rejection.

72. A method according to any one of claims 57 to 71, wherein the progeny are differentiated *ex vivo* or *in vivo*.

73. A method according to claim 72, wherein the progeny are selected from the group consisting of osteoblasts, chondrocytes, adipocytes, fibroblasts, marrow stroma, skeletal muscle, smooth muscle, cardiac muscle,

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ocular endothelial, epithelial, hepatic, pancreatic, hematopoietic, glial, neuronal and oligodendrocytes.

74. A method according to any one of claims 57 to 5 73, wherein the mesenchymal connective tissue-derived stem cells or their progeny home to one or more organs in the animal and are engrafted therein such that the function of a cell or organ, defective due to injury, genetic disease, acquired disease or iatrogenic treatments, is augmented, 10 reconstituted or provided for the first time.

75. A method according to claim 74, wherein the disease is selected from the group consisting of cancer, cardiovascular disease, metabolic disease, liver disease, 15 diabetes, hepatitis, hemophilia, degenerative or traumatic neurological conditions, autoimmune disease, genetic deficiency, connective tissue disorders, anemia, infectious disease and transplant rejection.

20 76. A method according to claim 74, wherein the injury is ischemia or inflammation.

77. A method according to claim 74, wherein the organ is selected from the group consisting of bone 25 marrow, blood, spleen, liver, lung, intestinal tract, eye, brain, immune system, circulatory system, bone, connective tissue, muscle, heart, blood vessels, pancreas, central nervous system, peripheral nervous system, kidney, bladder, skin, epithelial appendages, breast-mammary 30 glands, fat tissue, and mucosal surfaces including oral esophageal, vaginal and anal.

78. A method according to any one of claims 57 to 77, wherein the mesenchymal connective tissue-derived stem 35 cells or their progeny are genetically transformed to deliver a therapeutic agent.

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79. A therapeutic composition comprising mesenchymal connective tissue-derived stem cells and a pharmaceutically acceptable carrier, wherein the mesenchymal connective tissue-derived stem cells are present in an amount effective to produce tissue selected from the group consisting of bone marrow, blood, spleen, liver, lung, intestinal tract, eye, brain, immune system, bone, connective tissue, muscle, heart, blood vessels, pancreas, central nervous system, kidney, bladder, skin, epithelial appendages, breast-mammary glands, fat tissue, and mucosal surfaces including oral esophageal, vaginal and anal.

80. A therapeutic method for restoring organ, tissue or cellular function to a mammalian animal in need thereof comprising the steps of: (a) removing mesenchymal connective tissue-derived stem cells from a mammalian donor; (b) expanding a mesenchymal connective tissue-derived stem cells to form an expanded population of undifferentiated cells; and (c) administering the expanded cells to the mammalian animal, wherein organ, tissue or cellular function is restored.

81. A method according to claim 80, wherein the function is enzymatic.

82. A method according to claim 80, wherein the function is genetic.

83. A method according to claim 80, wherein the mammalian donor is the patient.

84. A method according to any one of claims 80 to 83, wherein the organ, tissue or cell is selected from the group consisting of bone marrow, blood, spleen, liver, lung, intestinal tract, eye, brain, immune system, bone, connective tissue, muscle, heart, blood vessels, pancreas,

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central nervous system, peripheral nervous system, kidney, bladder, skin, epithelial appendages, breast-mammary glands, fat tissue, and mucosal surfaces including oral esophageal, vaginal and anal.

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85. A method of inhibiting the rejection of a heterologous mesenchymal connective tissue-derived stem cells transplanted into a patient comprising the steps of: (a) introducing into the mesenchymal connective tissue-derived stem cells, *ex vivo*, a nucleic acid sequence encoding the recipient's MHC antigens operably linked to a promotor, wherein the MHC antigens are expressed by the mesenchymal connective tissue-derived stem cells; and (b) transplanting the mesenchymal connective tissue-derived stem cells into the patient, wherein MHC antigens are expressed at a level sufficient to inhibit the rejection of the transplanted mesenchymal connective tissue-derived stem cells.

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86. A method according to claim 85, wherein the patient is of the same species or another mammalian species as the donor of the mesenchymal connective tissue-derived stem cells.

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87. A method according to claim 85, wherein the mesenchymal connective tissue-derived stem cells are transplanted into the patient via localized injection, systemic injection, parenteral administration, oral administration, or intrauterine injection into an embryo.

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88. A method according to claim 87, wherein localized injection comprises catheter administration.

89. A method according to any one of claims 85 to 88, wherein cells are transplanted in conjunction with a pharmaceutically acceptable matrix.

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90. A method according to claim 89, wherein the matrix is biodegradable.

91. A method of nuclear transfer comprising the step
5 of transferring a mesenchymal connective tissue-derived stem cell or nuclei isolated from a mesenchymal connective tissue-derived stem cell into an enucleated oocyte.

92. A method for producing a genetically engineered
10 or transgenic non-human mammal comprising:

(i) inserting, removing or modifying a desired gene in a mesenchymal connective tissue-derived stem cell from a non-human mammal or nuclei isolated from a mesenchymal connective tissue-derived stem cell isolated
15 from a non-human mammal; and

(ii) transferring the a mesenchymal connective tissue-derived stem cell or nuclei into an enucleated oocyte.

93. A method for producing a genetically engineered
20 or transgenic non-human mammal comprising:

(i) inserting, removing or modifying a desired gene or genes in a mesenchymal connective tissue-derived stem cell from a non-human mammal or nuclei isolated from
25 a mesenchymal connective tissue-derived stem cell isolated from a non-human mammal; and

(ii) inserting a mesenchymal connective tissue-derived stem cell or nuclei into an enucleated oocyte under conditions suitable for the formation of a
30 reconstituted cell;

(iii) activating the reconstituted cell to form an embryo;

(vi) culturing said embryo until greater than the 2-cell developmental stage; and

35 (v) transferring said cultured embryo to a host mammal such that the embryo develops into a transgenic fetus.

94. A method for cloning a non-human mammal comprising:

- 5 (i) inserting a mesenchymal connective tissue-derived stem cell from a non-human mammal or nuclei isolated from a mesenchymal connective tissue-derived stem cell isolated from a non-human mammal into an enucleated mammalian oocyte, under conditions suitable for the formation of a reconstituted cell;
- 10 (ii) activating the reconstituted cell to form an embryo;
- (iii) culturing said embryo until greater than the 2-cell developmental stage; and
- 15 (iv) transferring said cultured embryo to a host mammal such that the embryo develops into a fetus.

95. A method according to any one of claims 91 to 94, wherein the oocytes are isolated from either oviducts and/or ovaries of live animals.

20 96. A method according to any one of claims 91 to 95, wherein the oocytes are enucleated oocytes and zona pellucida-free.

25 97. A method according to claim 96, wherein the step of removing the zona pellucida is by a method selected from the group consisting of physical manipulation, chemical treatment and enzymatic digestion.

30 98. A method according to claim 96, wherein the step of removing the zona pellucida is by enzymatic digestion.

35 99. A method according to claim 98, wherein the enzyme used to digest the zona pellucida is a protease, a pronase or a combination thereof.

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100. A method according to claim 99, wherein the enzyme is a pronase.

5 101. A method according to claim 99, wherein the enzyme is a pronase.

102. A method according to claim 99, wherein the enzyme is a pronase.

10 103. A method according to claim 100, wherein the pronase is used at a concentration between 0.1 to 5%.

104. A method according to claim 100, wherein the pronase is used at a concentration between 0.25% to 2%.

15 105. A method according to claim 100, wherein the pronase is used at a concentration of about 0.5%.